Veterinarsky Obzor,
Vol. 32-33; 1939;
pp. 499-502

O VLIVU PIKLU NA KUŽE INFIKOVANÉ SNĚTÍ SLEZINNOU

THE INFLUENCE OF PICKLING ON SKINS INFECTED WITH ANTHRAX

By

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Skins suspected of being infected with anthrax must be treated, when technically processed, with methods that will devitalize any anthrax spores that might be present. Pickling is the method that is usually applied for this purpose. This should be done with ever increasing care - the skins should be stamped to distinguish those of European origin from those that are imported from other continents, since we do not have sufficient health guarantees when it comes to skins of foreign origin. We must also remember that skins of domestic animals are more and more widely used. Nowadays large quantities of skins are also used for gelatin, industrial gut, etc., and the raw material for these products is equally imported. The decontamination of skins for food industry purposes has this advantage that the skins used in this instance - as opposed to leathers used for shoes - come in fairly small pieces. This makes it much easier to handle the skins while they are being disinfected. Another advantage is that skins used for food products do not have to be kept whole while being processed. This allows us to apply techniques that could not

be used on shoe leather.

The Seymour-Jones and the Schattenfroh method are two of the most commonly used methods for devitalising the anthrax spores. The first one is not suitable for skins that go into food products since it involves the use of sublimate. The second one uses hydrochloric acid and sodium chloride (for experiments with this method see: Sevcik F., Zschr. f. Infkr. H., XIII, no. 6, 7 and Wien. tierärtstl. M., I, p. 127). We took it for our task to test this method again, and eventually to improve it as far as the devitalization of the anthrax spores is concerned.

In our experiments we used only the type of skins that is used for food produce. The experiments that are described on the following pages have been selected from a series made in connection with this study.

Experiment 1.

The pickle that we used had the following composition: Hydrochloric acid with the specific strength of 1.19; 300 ccm of water; 115 g. of sodium chloride. The pickle must have 13 Bé.

There were 8 strains of Bac. anthracis in all. A certain percent of them showed a good, and even an excellent sporogeny. It was known that the strain had a high virulence thanks to the preceding official tests that had been made with it. The spores were prepared in such a way that cultures made on grossed agar were kept at a temperature

of 37 C until an adequate number of spores could be found in the microscopic preparations. This took, as a rule, 14 days. Following this the spores were washed down with water and the very heavy suspension was either thickly injected into the skins (about one puncture to every 1 ccm) or used directly for pickling as shown under heading A).

A) The influence of the pickle on the spores.

Method: 45 ccm of hydrochloric acid and 115 g of sodium chloride was added to 300 ccm of adequately infected spore suspension. The pickle was put into a container with a ground joint stopper, and left to stand over night in the laboratory. During the day the container was shaken from time to time.

Results: After 14, then after 24 hours of interaction, 6 dishes of ordinary agar were inoculated with this fluid. After incubation the sediment was tested and found to be sterile.

B) The influence of the pickle on infected skins.

The skins (vaccines) that 2 days previously had been infected with the suspension of spores (a thick growth had been ascertained in the cultures) were covered with the pickling fluid so that there was 45 ccm of hydrochloric acid, 300 ccm of water, and 115 g of sodium chloride to every kilogram of skins. The skins and the pickling mixture were put in a glass container with a ground joint stopper, and for 12 hours were stirred by rotating the container. 4 hours later 10 of the skins were inoculated in the following manner:

part of the skins was transferred directly into a culture medium, the other part was washed with a weak solution of sodium carbonate, then with water, and finally put into a culture medium. In this way a specific growth was obtained from 7 of the skins. This growth was identified by the staining properties of its colonies, and by the results it gave in animal tests. In the pickling fluid in which the skins had been processed Bac. anthracis was equally grown.

Conclusions: The pickle, the concentration of which did not change in the course of the experiment (experiment A), destroys anthrax spores within 14 hours already. In the pickling mixture that had been poured over the skins (in this fluid the concentration is lowered due to the water contained in the skins) some spores of Bac. Anthracis remain alive but we can say, judging by the growth in the dishes, that only a small percent of them remains alive after this treatment.

Experiment 2.

In this experiment the pickling mixture was composed of the following ingredients: 2.5 liters of hydrochloric acid at the specific strength of 1.19, 5 liters of water, and 5 kg of commercial sodium chloride. The strains used in this experiment were the same as in experiment #1. In addition to the 8 strains used previously we used also cultures of the colonies that had been grown from skins in the previous test which had also been put through mouse passages. Both the suspension, and the infecting of the skins with this suspension

were carried out exactly like in the previous experiment. Two mice that were inoculated with 0.2 ccm of this suspension died two days later. The autopsy, the bacterioscopic, bacteriological and serological examinations revealed typical anthrax findings.

A) The influence of the pickle on the spores.

Method: 1 kg of sodium chloride and 1 liter of a thick spore suspension were added to 0.5 l. of HCl. The mixture was shaken from time to time in a container with a ground joint stopper. After 2, 4, 6, 8, 10 and 24 hours 0.5 ccm samples of fluid were taken from the container. The samples were neutralized with lye and put into dishes containing respectively 0.16, 0.08, 0.04, and 0.02 ccm of ordinary agar. With the samples that were taken after 2 and 4 hours 4 dishes were inoculated each time, and later on, 6 dishes. All of the plates were first incubated for 24 hours at a temperature of 37 C, then for two days at room temperature. When checked they showed no specific growth.

B) The influence of the pickle on the skins.

50 kg of the infected, inoculated skins - each piece about 1 cm thick, and measuring approximately 15 x 25 cm - were put in a vat and covered with the following pickling fluid: 2.5 liters of hydrochloric acid, 5 kg of sodium chloride, and 5 liters of water. The vat was gently rotated for 13 hours. This process completed, the skins were taken out and allowed to drain on a wooden grate.

In due time samples the size of a hazel nut were taken from the various skins. These samples were cut into small pieces and neutralised with a 0.1% solution of soda. Then the material from each separate skin was transferred into 3 Petri dishes. Ordinary agar was added to these dishes, whereupon they were incubated for 24 hours at 37C, and for 2 days at a temperature of approximately 20 C. Following which the check was made.

Results: After 13 hours of pickling (thus, immediately after taking the material from the vat) samples were taken from 3 skins. When tested they were found to be sterile. The next day samples were taken from 8 skins. These were ground and put into agar without sodium chloride. The pH was brought to 7.2 with soda solution, and they were poured into 21 dishes. No specific growth was found in them. The same day pieces were taken from 8 skins. The samples were ground, neutralized with soda solution, mixed, and introduced into the abdominal cavity of two guinea pigs after a laparotomy. The animals remained healthy. After 14 days samples were taken from 32 skins. They were ground and transferred to 1 liter of plain but unsalted agar, then the pH was adjusted to 7.2 with soda solution. All in all, 86 dishes were filled but not one of them showed a sign of the specific growth.

Conclusions: The results of this experiment prove that it was possible to devitalize the spores by increasing the acidity of the pickle and

augmenting the amount of the pickling fluid per kilogram of the material.

Summary.

In view of the fact that the food industry is steadily expanding we must apply greater care indovitalizing the spores of Bac. anthracis in skins. For this reason; namely, that skins are used in the manufacture of gelatin and other food products of a gelatin-like nature, and that the skins that are used for the purpose are often of foreign origin.

We have studied two different concentrations of pickle and the influence that these concentrations as well as the amount of pickling fluid used per 1 kg of material has on the skins. We have established that a pickle that consists of 0.5 l of hydrochloric acid, 1 kg of sodium chloride, and 1 liter of water used for each kg of skins effectively destroys the spores of Bac. anthracis.

Translated at the National Institutes of Health
Bethesda, Maryland
Nov. 30, 1949

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